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EXPEDITED PROCEDURE - RESPONSE AFTER FINAL

DATE: November 10, 2003

FROM: Kathleen D. Rigaut, Ph.D., J.D.

DELIVER TO: Examiner Li
Art Unit 1632
Fax number (703) 872-9306

RE: U.S. Patent Application No. 09/487,851

Total Pages (including this cover) 43

Examiner Li:

As per our telephone conference I am faxing a copy of a Rule 131 Declaration in connection with the outstanding official action in the above-identified patent application.
Thank you for your attention to this matter.

Respectfully submitted,
Kathleen D. Rigaut, Ph.D., J.D.
Reg. No. 43,047

IF THE ACCOMPANYING/ABOVE MESSAGE IS NOT RECEIVED PROPERLY, PLEASE CALL (215) 563-4100 AND LEAVE A MESSAGE FOR THE OPERATOR INDICATED ABOVE.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of	)	Group Art Unit: 1632
Robert J. Levy et al.	)	Examiner: Li, Qian J
Serial No. 09/487,851	)	Response to Paper No.29
Filed: January 19, 2000	)	
For: "Reverse Gene Therapy"	)	

DECLARATION OF ROBERT J. LEVY

I, Robert J. Levy, hereby declare that:

1. I am a citizen of the United States and reside at 440 Merion Road, Merion Station, PA 19066.

2. I received a Bachelor's degree from Washington University and an M.D. from Johns Hopkins School of Medicine. The details of my education and professional history are set forth in my curriculum vitae, attached hereto as Exhibit A.

3. I have over 33 years experience in the field of medicine, my particular area of expertise being in cardiovascular disease.

4. I am the author or co-author of more than 145 scientific articles on the subjects of gene therapy, cardiac therapy, and heart valve disease. A list of these articles is set forth in my curriculum vitae, attached hereto. My current area of research involves utilizing reverse gene therapy to treat cardiac arrhythmias. I have discovered that administration of a hMiRP1 ion channel mutant has a positive

therapeutic electrophysiologic effect that could be used in treating re-entrant atrial flutter.

5. I am an inventor of the subject matter disclosed and claimed in U.S. Patent Application Serial No. 09/487,851, entitled "Reverse Gene Therapy." (hereinafter "the '851 application").

Statements Regarding Adequacy of the Disclosure to Enable Practice of the Invention

6. I have read and am familiar with the Official Action dated February 27, 2003, in the '851 application. I understand the nature of the rejection made by the Examiner concerning adequacy of the disclosure to enable one skilled in the art to practice the invention.

7. As exemplified in the specification, I have discovered that administration of a mutant HERG gene to the cardiac tissue produces delayed cardiac repolarization. Various means of practicing this method, as well as evidence of it's efficacy are disclosed throughout the specification. For example, pages 11-13 of the specification discuss the above gene, and mutants thereof (Q9E-hMiRP1), which are effective in preventing re-entrant atrial flutter. Similarly, page 13 of the specification describes the Q9E-hMIRP mutation in the MIRP gene, which interferes with the physiological function of HERG. When a MIRP mutant is provided to the atrial myocardium of a subject with re-entrant atrial flutter, the conductivity of the atrial tissue would be thereby decreased, and the disorder is alleviated.

Various means of administering these mutant HERG genes are discussed throughout the specification, and are specifically described at pages 19-23 of the specification. Additionally, Example III of the specification provides detailed methods of administering a mutant hMiRP1 gene to achieve the equivalent of Class III anti-arrhythmic activity.

8. Further evidence of the efficacy of administration of mutant hMiRP1 to treat re-entrant atrial flutter is provided herewith as Exhibit B. This evidence demonstrates that a mutant (Q9E-hMiRP1) transgene can be used to mimic class III anti-arrhythmic effects, and that these effects can be limited to a specific area of the atrial myocardium to disrupt regional re-entrant arrhythmia pathways.

Individuals who carry the Q9E-hMiRP1 variant exhibit diminished potassium currents, resulting in delayed myocardial repolarization following clarithromycin administration.

Exhibit B demonstrates that administration of a gene therapy vector comprising the Q9E-hMiRP1 variant to the atrial myocardium, followed by clarithromycin injection induces waveform changes and prolongation of the atrial epicardial monophasic action potential (MAP) duration. The MAP duration increases with length of clarithromycin administration in Q9E mutant but not wild type pigs.

The hMiRP1 and Q9E-hMiRP1 plasmids were created by subcloning the full-length coding sequence of the hMiRP1 potassium channel and the missense mutation, Q9E-hMiRP1 into the BAMHI/SACI sites of the pIRES2-eGFP bicistronic expression vector from Stratagene (LaJolla, CA). DAC heteroplexes were generated using an optimized formulation consisting of 10 mg of GFP plasmid DNA ("D") mixed with 10mg of mouse monoclonal anti-

bovine DNA IgM (U.S. Biological, Swampscott, MA) ("A") in a total volume of 50 μl PBS, followed by incubation at 37°C for 1 hour. 5 ml of cationic lipid ("C"), composed of a 1:1 (w/w) formulation of N-[1-(2,3-dioleyloxy)propyl]-n,n,n-triethylammonium chloride (DOTMA, Sigma Chemical Co., St.Louis, MO) and dioleoyl phosphatidylethanolamine (DOPE, Sigma) was added to DA with vortexing to form DAC. The heteroplex (DAC) was incubated at room temperature for 35 minutes or more before use.

However, initial DNA injection studies used only plasmid DNA ("naked DNA", uncomplexed) in pig atrial myocardial injection studies using hMiRP1, or Q9E-hMiRP1 plasmids. Following a right thoracotomy under general anesthesia, a series of pigs were subjected to atrial myocardial injections with DAC preparations using either hMiRP1, or Q9E-hMiRP1 plasmids. Then the waveform changes were measured at various time points after clarithromycin infusion. (See attached protocol and figures) Q9E-MiRP1 transfection plus clarithromycin was the model therapeutic approach investigated in these studies, because of the comparable mechanisms of action to Class III anti-arrhythmics, that also result in diminished potassium channel currents. Therefore, the I_{Kr} response of transgene Q9E-hMiRP1 to clarithromycin demonstrated in the present studies may be used to control regional atrial re-entrant arrhythmia activity. This strategy is also attractive since the electrophysiologic effects of over expressed Q9E-hMiRP1 can be modulated with variable dosing of clarithromycin or its analogues. Additionally, other potassium channel mutations such as the dominant negative HERG mutation, A561V, should also yield promising results as candidate gene therapy constructs. A brief summary of these

methods is provided in Exhibit B.

9. Taken together, the teachings in the specification, and these experimental results clearly demonstrate the adequacy of the disclosure of the '851 application to enable anyone skilled in the art to practice the methods of the claimed invention without undue experimentation.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so make are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the above-referenced application or any patent issued thereon.

11/8/03
DATE

Robert J. Levy

R. Levy
Septebmer 2003
CONFIDENTIAL

CURRICULUM VITAE

Robert J. Levy, M.D.

Home Address: 440 Merion Road
Merion, PA 19066

Office Address: The Children's Hospital of Philadelphia
Abramson Pediatric Research Center, Room 1107B
34th & Civic Center Boulevard
Philadelphia, PA 19104-4399

Social Security Number: 498-46-1620

Education:

1966	B.A.	Washington University, St. Louis
1970	M.D.	Johns Hopkins University School of Medicine, Baltimore
1998	M.A.	University of Pennsylvania (Honorary)

Postgraduate Training and Fellowship Appointments:

1970-71	Intern, Children's Hospital of Pittsburgh, Pittsburgh
1971-73	Resident, The Johns Hopkins Hospital, Department of Pediatrics, Baltimore
1975-78	Fellow in Cardiology, Children's Hospital Medical Center, Boston
1975-78	Clinical Fellow in Pediatrics, Harvard Medical School, Boston

Military Service:

1973-75	Lieutenant Commander, Medical Corps, U.S. Navy, Chief of Pediatrics, Naval Hospital, Portsmouth, New Hampshire

Faculty Appointments:

1978-80	Instructor in Pediatrics, Department of Pediatrics, Harvard Medical School
1978-80	Assistant in Cardiology, Department of Cardiology The Children's Hospital, Boston
1980-86	Associate in Cardiology, Department of Cardiology The Children's Hospital, Boston
1980-86	Assistant Professor of Pediatrics, Department of Pediatrics, Harvard Medical School
1981-86	Associate in Cardiology, Laboratory of Human Biochemistry, The Children's Hospital, Boston
1986-97	Associate in Cardiology, Department of Pediatrics, University of Michigan Medical School, C.S. Mott Children's Hospital
1986-90	Associate Professor of Pediatrics, Department of Pediatrics, University of Michigan Medical School
1986-91	Associate Professor of Pharmaceutics, Department of Pharmaceutics University of Michigan School of Pharmacy

1990-97	Professor of Pediatrics, Department of Pediatrics University of Michigan Medical School
1991-97	Professor of Pharmaceutics, Department of Pharmaceutics, University of Michigan Medical School
1997	Senior Member, Joseph Stokes Research Institute, The Children's Hospital of Philadelphia
1997	Member, Institute for Medicine and Engineering, University of Pennsylvania
1997	Member, Institute for Human Gene Therapy, University of Pennsylvania
1997	Professor of Pediatrics, Tenure Track, The University of Pennsylvania School of Medicine
1998	The William J. Rashkind Chair in Pediatric Cardiology, The Children's Hospital of Philadelphia
1999	Professor of Pharmacology, The University of Pennsylvania School of Medicine

Hospital and Administrative Appointments;

Harvard Medical School:

1983-86	Preventive Cardiology Clinic (Director), The Children's Hospital, Boston

University of Michigan

1986	Director, Pediatric Cardiology Biochemistry Laboratories
1987-92	Research Advisory Committee, Department of Pediatrics
1988-91	Faculty Senate
1988-89	Chairman, Search Committee for the Directorship of Pediatric Neurology
1991	Office of the Vice President for Research's (OVPR) Advisory Committee on Improving the Quality and Cost Effectiveness of OVPR's Operations

Other

1995	Tenure Review Committee, The Hebrew University of Jerusalem

The Children's Hospital of Philadelphia

1997	Senior Physician
1997	Senior Member, Joseph Stokes Jr. Research Institute
2001	Director, Cardiology Research Laboratories, Children's Hospital of Philadelphia

Specialty Certification:

1975	American Board of Pediatrics

Licensure:

1986	Michigan
1997	Pennsylvania

Awards, Honors and Membership in Honorary Societies:

1965 Phi Eta Sigma
1966 Phi Beta Kappa
1982 American Academy of Pediatrics Young Investigator Award
1983 Society for Pediatric Research
1985 Whitaker Health Sciences Foundation Award
1986 Established Investigator of the American Heart Association
1987 Rackham International Fellowship with The Hebrew University of Jerusalem
1987-90 Investigator, United States — Israel Binational Science Foundation
1988 Ebert Prize of the American Pharmaceutical Association
1988 American Pediatric Society
1990 Alpha Phi Foundation Cardiovascular Research Prize
1992 Clemson Award, Society for Biomaterials
1994 Fellow, National Academy of Biomaterials Science and Engineering
1995 Forchheimer Sabbatical Professor, The Hebrew University of Jerusalem
1995 University of Michigan Technology Award
1996 University of Michigan Technology Award
1996 Honorary Professorship, Institute of Biomedical Engineering of Peking Union Medical College and Chinese Academy of Medical Sciences
1996 Honorary Professorship, Cardiovascular Institute and Fu Wai Hospital, Chinese Academy of Medical Sciences
1998 Fellow, American Institute for Medical and Biological Engineering
2000 Member, John Morgan Society, University of Pennsylvania School of Medicine
2001 Discover Magazine Technology Award
2002 Children's Hospital of Philadelphia Technology Award
2002 Luigi Mastroianni Clinical Innovator Award, University of Pennsylvania School of Medicine
2002 Johnson and Johnson Focused Giving Program Award

Memberships in Professional and Scientific Societies:

National Societies:

American Society for Artificial Internal Organs, Program Committee (1994)
World Congress Program Committee, International Society for Heart Research (1987-1989)
Second Jerusalem Conference on Pharmaceutical Sciences, Planning Committee (1995)
Controlled Release Society, International Program Committee, Nice, France (1994)
Third Jerusalem Conference on Pharmaceutical Sciences, Symposium Co-Chair (1995-96)
Executive Committee, International Society for Applied Cardiovascular Biology (1996-98)
Executive Board, American Society for Artificial Organs (1996-98)
4th Jerusalem Conference on Pharmaceutical Sciences, International Program Committee (1999)
Member at Large, Executive Board, Society for Biomaterials (2001-02)

Local Societies:

None

Research Grant Review Activity (Selected)

1993	Site Visit Reviewer, Medical Research Council of Canada
1993	NHLBI Study Section on Cardiovascular Disease in Women
1994	Ad Hoc Reviewer, NIH Special Study Section on Biomaterials
1998	Member, NIH Special Study Section on Tissue Engineering and Biomimetics
2001	National Institutes of Health, Training Grant Study Section
2001	National Science Foundation, Special Study Section on Retroviral Gene Therapy Vectors
2001	National Institutes of Health, Special Study Section on Centers of Biomedical Research Excellence
2001	National Institutes of Health, Chair, Special Study Section on Cardiovascular Calcification
2001	Ad Hoc Member, NIH Pathology A Study Section.
2001	Member NHLBI Training Grant Study Section.
2002	Member, NIH Special Study Section on Tissue Engineering.
2003	Member, NHLBI Program Project Special Emphasis Panel
2003	Member, NIH Special Study Section on Tissue Engineering
2003	Member, Special Review Panel, Irish National Science Foundation
2003	External reviewer, Medical Research Council of Canada

Business Development Activities:

1995	Co-Founder, *Selective Genetics, Inc.* San Diego, CA

Editorial Board Positions:

1992	Editorial Board, Biomaterial-Living System Interactions (BIOMIR), Moscow
1996-98	Editorial Board, ASAIO Journal
1996	Guest Editor, Advanced Drug Delivery Reviews
1998	Editorial Board, Biomaterials
1999	Editorial Board, Pharmaceutical Research

Editorial Board Reviewing Activity

American Journal of Pathology
Annals of Thoracic Surgery
ASAIO Journal
Biomaterials
Cancer Research
Cardiovascular Pathology
Circulation
Circulation Research
FASEB Journal
Gene Therapy
Human Gene Therapy

Journal of Biomaterial Science: Polymer Edition
Journal of Cardiovascular and Thoracic Surgery
Journal of Cardiovascular Pathology
Journal of Clinical Investigation
Journal of Heart Valve Disease
Journal of Microencapsulation
Journal of Pharmaceutical Science
Molecular Therapy
Nature Biotechnology
Pharmaceutical Research
Proceedings of the National Academy of Sciences
The Journal of Biomedical Materials Research
The Journal of Controlled Release

Academic Committees at the University of Pennsylvania and The Children's Hospital of Philadelphia:

1998	Member, Committee on Appointments and Promotions, The Children's Hospital of Philadelphia
1998	Member, Stokes Lectureship Committee, The Children's Hospital of Philadelphia
1998	Member, I.R. B., The Children's Hospital of Philadelphia
1999	Program Director, NHLBI Institutional Research Service Award, Molecular Therapeutics for Pediatric Cardiology
1999	Member, Committee on Fetal Therapy, The Children's Hospital of Philadelphia
1999	Member, Medical Advisory Committee for the Foerderer Fund for Excellence, The Children's Hospital of Philadelphia
1999	Medical Advisory Subcommittee for the Foerderer Fund
2000	Member, Biomedical Coordination Committee (BEN@ PENN) University of Pennsylvania
2000	Chair, Committee on Appointments and Promotions, The Joseph Stokes Jr. Research Institute, The Children's Hospital of Philadelphia
2002	Oversight Committee, NIH Clinical Trial for Twin-Twin Transfusion
2002	Oversight Committee, NIH Clinical Trial for Fetal Meningomyclocele Repair

Academic Committees at the University of Michigan:

1986-97	Pediatric Cardiology Biochemistry Laboratories (Director)
1986-97	Attending Physician, Pediatric Cardiology
1987-92	Research Advisory Committee, Department of Pediatrics
1988-91	Faculty Senate
1988-89	Chairman, Search Committee for the Directorship of Pediatric Neurology
1988-91	University Committee on the Use and Care of Animals (UCUCA)
1989-92	Biomedical Research Council
1989-92	Director, Preventive Cardiology Clinic, C.S. Mott Children's Hospital
1990-92	Medical Student Fellowship Committee

1990-97	Pediatric Preventive Cardiology Clinic (Director)
1990-93	Board for Student Publications
1991	Office of the Vice President for Research's (OVPR) Advisory Committee on Improving the Quality and Cost Effectiveness of OVPR's Operations
1991	Department of Pediatrics First Annual Research Symposium
1991	Evaluation and Management of Valvular Insufficiency: New Approaches for the 90's, Department of Internal Medicine
1991	The Restenosis Summit III, Department of Internal Medicine
1991	Cardiovascular Research Center
1991	Newborn Care Internal Review Committee, Department of Pediatrics
1992	Search Committee for Chair of Biomaterials, Dental School
1992-93	Search Committee, Chief of Newborn Services, Department of Pediatrics
1992-93	Fund Raising Committee, Amnon Rosenthal Professorship, School of Medicine
1994	SCOR (NIH) in Rheumatoid Arthritis Internal Advisory Board
1995	Child Health Research Center Advisory Committee
1995	Materials Science Center Internal Advisory Committee, Office of the Vice President for Research
1995	Tissue Engineering Working Group, School of Medicine
1996	Michigan Congenital Heart Center Coordinating Council
1996	Research Advisory Council
1996	Search Committee, Pediatric Pulmonology
1996	Medical School Committee on Student Biomedical Research

Major Teaching and Clinical Responsibilities at the University of Michigan

1986-1997 Attending Physician, Pediatric Cardiology, University of Michigan Hospitals

Lectures by Invitation (Since 1994):

1994	"Polyurethane Calcification" — American Chemical Society Polymer Symposium, Ann Arbor, Michigan
1994	"Controlled Release for Arrhythmias" — Third European Symposium on Controlled Drug Delivery, The Netherlands
1994	"Clinical Controlled Release Systems" — Twenty-First International Symposium on Controlled Release of Bioactive Materials, Nice, France
1994	"Cardiac Valve Bioprosthesis" — ASAIO/NIH Cardiovascular Science and Technology Conference, Washington, D.C.
1995	Invited Participant, National Heart, Lung and Blood Institute Workshop on Tissue Engineering, held at 1995 Gordon Conference on Biomaterials, Holderness, New Hampshire
1996	"Mechanistic Approaches for Preventing Bioprosthetic Calcification" — International Society for Applied Cardiovascular Biology Fifth Biennial Meeting, Manchester, England
1996	"Polymers in Medicine" — Academy of Medical Sciences, People's Republic of China
1996	"Advanced Therapies for Cardiac Valve Disease" — National Heart, Lung and Blood Institute Workshop on Heart Valve Prostheses, Washington, D.C.

1996	"How to Prevent or Mitigate Dystrophic Calcification" — XVIII Congress of the European Soci ty of Cardiology, Birmingham, England
1996	"Cardiac Controlled Release Implants for Arrhythmias" — Third Jerusalem Conference on Pharmaceutical Sciences and Clinical Pharmacology, Jerusalem, Israel
1996	"Cardiac Drug Delivery Mechanisms" — Cardiology Grand Rounds, The University of Michigan, Ann Arbor, Michigan
1997	"Current Progress in Anticalcification for Bioprosthetic and Polymeric Heart Valves" — The University of Michigan Medical School, Ann Arbor, Michigan
1997	"Differential Calcification of Cusps and Aortic Wall of Failed Stented Porcine Bioprosthetic Valves" — The University of Michigan Medical Center, Ann Arbor Michigan
1997	"Synergistic Inhibition of Calcification of Porcine Aortic Root with Preincubation in FeC13 and Alpha-Amino Oleic Acid in a Rat Subdermal Model, Medtronic Heart Valve, Irvine, CA.
1997	"Arterial Nanoparticle Administration for Restenosis", Presented at the 24th International Symposium on Controlled Release of Bioactive Materials Stockholm, Sweden
1997	"Valvular Drug Delivery", Presented at the Conference on Formulations and Drug Delivery, sponsored by the American Chemical Society and the Controlled Release Society, La Jolla, California
1997	"Sustained Release Nanoparticles for Restenosis", Presented at the American Association of Pharmaceutical Science, Boston
1997	"Delivery Enhancers', 4th European Conference on Cardiovascular Drug Delivery, Geneva, Switzerland.
1997	"Technological Advances for Prosthetic Heart Valves", Shaping the Future of Cardiac Surgery, Paris, France.
1998	"Anticalcification Treatment: State of the Art" Endocarditis and Thrombogenecity in Patients with Prosthetic Valves, Helsinki, Finland
1999	Bioprosthetic Heart Valve Calcification: Mechanisms and Prevention, Epic Heart Valve Clinical Launch, Ivalo, Finland
1999	Clinical use of the Ethanol Pretreated Bioprosthesis, BioCor Institute Belo Horizonte, Brazil.
2000	"Mechanisms of Cardiovascular Calcification". Pharmacology Seminar, University of Pennsylvania School of Medicine
2000	"Controlled Release Stents". Lifeline Foundation, Washington, D.C.
2000	"Gene Delivery Systems" XII International Symposium on Atherosclerosis. Stockholm, Sweden.
2001	Calcification Resistance with Aluminum-Ethanol Treated Porcine Aortic Valve Bioprostheses. Stentless Heart Valve Meeting, San Diego.
2001	Inhibition of Cusp and Aortic Wall Calcification in Ethanol and Aluminum Treated Heart Valves in Sheep. Society for Heart Valve Disease, London, United Kingdom.
2001	Delivery Stents. Boston Scientific. Natick, Massachusetts
2001	Bench Research in the 1970's. The Alexander Nadas Memorial Symposium.

Children's Hospital, Boston

2002 Heart Valve Disease, UWEB Symposium, University of Washington, Seattle

2002 Gene Delivery Systems, Genzyme Corporation, Boston, Massachusetts

2002 Antibody-mediated Gene Delivery, Cystic Fibrosis Research Foundation, Philadelphia, PA

2002 Reverse Gene Therapy, Johnson & Johnson, New Brunswick, NJ

2003 Site Specific Gene Therapy, National Institutes of Standards and Technology, Gaithersburg, MD

2003 TGA Preclinical Strategies: St. Jude Medical, St. Paul, MN

Bibliography:

Research Publications, peer reviewed:

1. Levy, R.J., Rosenquist, G.C.: Anatomical variations in tricuspid atresia: report of two cases with previously undescribed lesions, Johns Hopkins Medical Journal 126:177-183, 1970.
2. Krovetz, L.J., Simon, A.L., Levy, R.J., Tift, W.: Effects of angiographic contrast media on left ventricular function, Johns Hopkins Medical Journal 127:172-179, 1970.
3. Rosenquist, G.C., Levy, R.J., Rowe, R.D.: Right atrial-left ventricular relationships in tricuspid atresia, American Heart Journal 80:493-500, 1970.
4. Levy, R.J., Rosenthal, A., Freed, M.D., Smith, C.D., Eraklis, A., Nadas, A.S.: Persistent pulmonary hypertension in an infant with congenital diaphragmatic hernia successfully managed with Tolazoline, Pediatrics 60:740-742, 1977.
5. Levy, R.J., Rosenthal, A., Fyler, D.C., Nadas, A.S.: Birthweight of infants with congenital heart disease, American Journal of Diseases of Children 132:249-257, 1978.
6. Levy, R.J., Rosenthal, A., Castaneda, A.R., Nadas, A.S.: Growth after surgical repair of d-transposition of the great vessels with intact ventricular septum, Annals of Thoracic Surgery 25:225-232, 1978.
7. Levy, R.J., Rosenthal, A., Miettinen, O.: Determinants of growth in patients with ventricular septal defect, Circulation 57:793-799, 1978.
8. Levy, R.J., Lian, J.B.: Gammacarboxyglutamate excretion and warfarin therapy, Clinical Pharmacology and Therapeutics 25:562-571, 1979.
9. Levy, R.J., Lian, J.B., Gallop, P.M.: Atherocalcin, a gammacarboxyglutamic acid containing protein from atherosclerotic plaque, Biochemical and Biophysical Research Communications 91, 41-49, 1979.
10. Levy, R.J., Zenker, J.A., Lian, J.B.: Vitamin K-dependent calcium binding proteins in aortic valve calcification, Journal of Clinical Investigation 65:563-566, 1980.
11. Sanders, S.P., Levy, R.J., Freed, M.D., Norwood, W.I., Castaneda, A.R.: Use of Hancock porcine xenografts in children and adolescents, American Journal of Cardiology 46:429-438, 1980.
12. Lian, J.B., Levy, R.J., Bernhard, W.F., Szycher, M.: LVAD mineralization and gammacarboxyglutamic acid containing proteins in normal and pathologically mineralized tissues, Transactions of the American Society of Artificial Internal Organs 27:683-689, 1981.
13. Fishbein, M., Levy, R.J., Ferrans, V.J., Dearden, L.C., Nashef, A., Goodman, A.P., Carpentier, A.: Calcification of cardiac valve bioprostheses. Biochemical, histologic, and ultrastructural observations in a subcutaneous implantation model system, Journal of Thoracic and Cardiovascular Surgery 83:602-609, 1982.
14. Levy, R.J., Gundberg, C.M., Scheinman, R.: The identification of the vitamin K-dependent bone protein osteocalcin as one of the carboxyglutamic acid containing proteins present in calcified atherosclerotic plaque and mineralized heart valves, Atherosclerosis 46:49-56, 1983.
15. Levy, R.J., Zenker, J.A., Bernhard, W.F.: Porcine bioprosthetic valve calcification in bovine left ventricle to aorta shunts: studies of the deposition of vitamin K-dependent proteins, Annals of Thoracic Surgery 36:187-192, 1983.

16. Levy, R.J., Schoen, F.J., Levy, J.T., Nelson, A.C., Howard, S.L., Oshry, L.J.: Biologic determinants of dystrophic calcification and osteocalcin deposition in glutaraldehyde-preserved porcine aortic valve leaflets implanted subcutaneously in rats, American Journal of Pathology 113:143-155, 1983.
17. Levy, R.J., Schoen, F.J., Howard, S.L.: Mechanism of calcification of porcine bioprosthetic aortic valve cusps: role of T-lymphocytes, American Journal of Cardiology 52:629-631, 1983.
18. Sherman, F.S., Schoen, F.J., Hawley, M., Nichols, J., Levy, R.J.: Collagen cross-links: a critical determinant of bioprosthetic heart valve calcification, Transactions of the American Society of Artificial Internal Organs XXX:577-581, 1984.
19. Levy, R.J., Hawley, M.A., Schoen, F.J., Lund, S.A., Liu, P.Y.: Inhibition by diphosphonate compounds of calcification of porcine bioprosthetic heart valve cusps implanted subcutaneously in rats, Circulation 71:349-356, 1985.
20. Schoen, F.J., Levy, R.J., Nelson, A.C., Bernhard, W.F., Nashef, A., Hawley, M.: Onset and progression of experimental bioprosthetic heart valve calcification, Laboratory Investigation 52:523-532, 1985.
21. Levy, R.J., Wolfrum, J., Schoen, F.J., Hawley, M.A., Lund, S.A., Langer, R.: Inhibition of calcification of bioprosthetic heart valves by local controlled-release diphosphonate, Science 228:190-192, 1985.
22. Nelson, A.C., Schoen, F.J., Levy, R.J.: SEM methodology for study of the pathophysiology of calcification in bioprosthetic heart valves, Scanning Electron Microscopy I:209-213, 1985.
23. Levy, R.J., Golomb, G., Wolfrum, J., Lund, S.A., Schoen, F.J., Langer, R.: Local controlled-release of diphosphonates from ethylenevinylacetate matrices prevents bioprosthetic heart valve calcification,
Transactions of the American Society of Artificial Internal Organs 31:459-463, 1985.
24. Levy, R.J., Schoen, F.J., Sherman, F.S., Nichols, J., Hawley, M.A., Lund, S.A.: Calcification of subcutaneously implanted type I collagen sponges: effects of formaldehyde and glutaraldehyde, American Journal of Pathology 122:71-82, 1986.
25. Golomb, G., Dixon, M., Smith, M.S., Schoen, F.J., Levy, R.J.: Inhibition of bioprosthetic heart valve calcification by sustained local delivery of Ca and Na diphosphonate via controlled release matrices, Transactions of the American Society of Artificial Internal Organs 32:587-590, 1986.
26. Levy, R.J., Howard, S.L., Oshry, L.J.: Carboxyglutamic acid (Gla) containing proteins of human calcified atherosclerotic plaque solubilized by EDTA: molecular weight distribution and relationship to osteocalcin, Atherosclerosis 59:155-160, 1986.
27. Schoen, F.J., Tsao, J., Levy, R.J.: Calcification of bovine pericardium used in cardiac valve bioprostheses: role of glutaraldehyde-modified structural components in bioprosthetic tissue mineralization, American Journal of Pathology 123:134-145, 1986.
28. Jonas, R.A., Schoen, F.J., Levy, R.J., Castaneda, A.R.: Biological sealants and knitted Dacron-porosity and histological comparisons of vascular graft materials with and without collagen and fibrin glue pretreatments, Annals of Thoracic Surgery 41:657-663, 1986.
29. Golomb, G., Langer, R., Schoen, F.J., Smith, M.S., Choi, Y.M., Levy, R.J.: Controlled release of diphosphonate to inhibit bioprosthetic heart valve calcification: dose-response and mechanistic studies, Journal of Controlled Release 4:181-194, 1986.

30. Golomb, G., Schoen, F.J., Smith, M.S., Linden, J., Dixon, M., Levy, R.J.: The role of glutaraldehyde-induced crosslinks in calcification of bovine pericardium used in cardiac valve prostheses, American Journal of Pathology 127:122-130, 1987.
31. Golomb, G., Dixon, M., Smith, M.S., Schoen, F.J., Levy, R.J.: Controlled release drug delivery of diphosphonates to inhibit bioprosthetic heart valve calcification: release rate modulation with silicone matrices via drug solubility and membrane coating, Journal of Pharmaceutical Sciences 76:271-276, 1987.
32. Levy, R.J., Schoen, F.J., Lund, S.A., Smith, M.S.: Prevention of leaflet calcification of bioprosthetic heart valves with diphosphonate injection therapy: experimental studies of optimal dosages and therapeutic durations, Journal of Thoracic and Cardiovascular Surgery 94:551-557, 1987.
33. Webb, C.L., Benedict, J.J., Schoen, F.J., Linden, J.A., Levy, R.J.: Inhibition of bioprosthetic heart valve calcification with covalently bound amino-propanehydroxydiphosphonate, Transactions of the American Society of Artificial Internal Organs 33:592-595, 1987.
34. Schoen, F.J., Kujovich, J.L., Webb, C.L., Levy, R.J.: Chemically determined mineral content of explanted porcine aortic valve bioprostheses: correlation with radiographic assessment of calcification and clinical data, Circulation 76:1061-1066, 1987.
35. Webb, C.L., Benedict, J.J., Schoen, F.J., Linden, J.A., Levy, R.J.: Inhibition of bioprosthetic heart valve calcification with aminodiphosphonate covalently bound to residual aldehyde groups, Annals of Thoracic Surgery 46:309-316, 1988.
36. Johnston, T.P., Schoen, F.J., Levy, R.J.: Prevention of calcification of bioprosthetic heart valve leaflet by Ca^{2+} diphosphonate pretreatment, Journal of Pharmaceutical Sciences 77:740-744, 1988.
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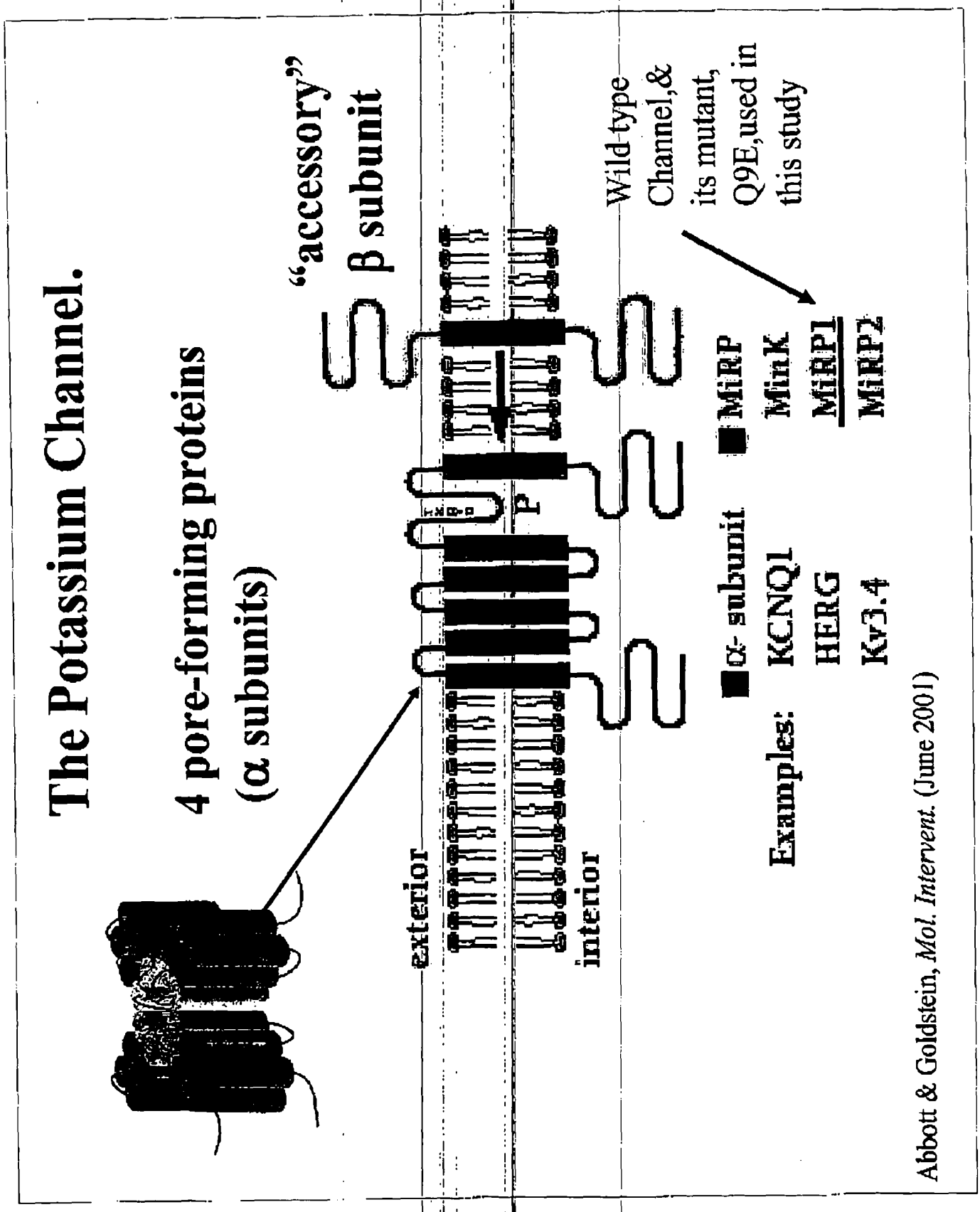
Background & Hypothesis:

- A missense ion channel mutation of Mink-related protein-1 (MiRP-1 Q9E) is associated with a clarithromycin dependent form of the Long QT syndrome (Abbott et al. *Cell* 99:175-87, 1999)
- Q9E results in prolonged inward K-rectifier currents (Ikr) compared to the wild type channel (WT), but only in the presence of the antibiotic clarithromycin. (Abbott et al. *Cell* 99:175-87, 1999)
- It is hypothesized that site specific overexpression in the atrium of Q9E with subsequent clarithromycin administration could result in controllable anti-arrhythmia gene therapy

The rationale for the use of Q9E Gene Vectors:

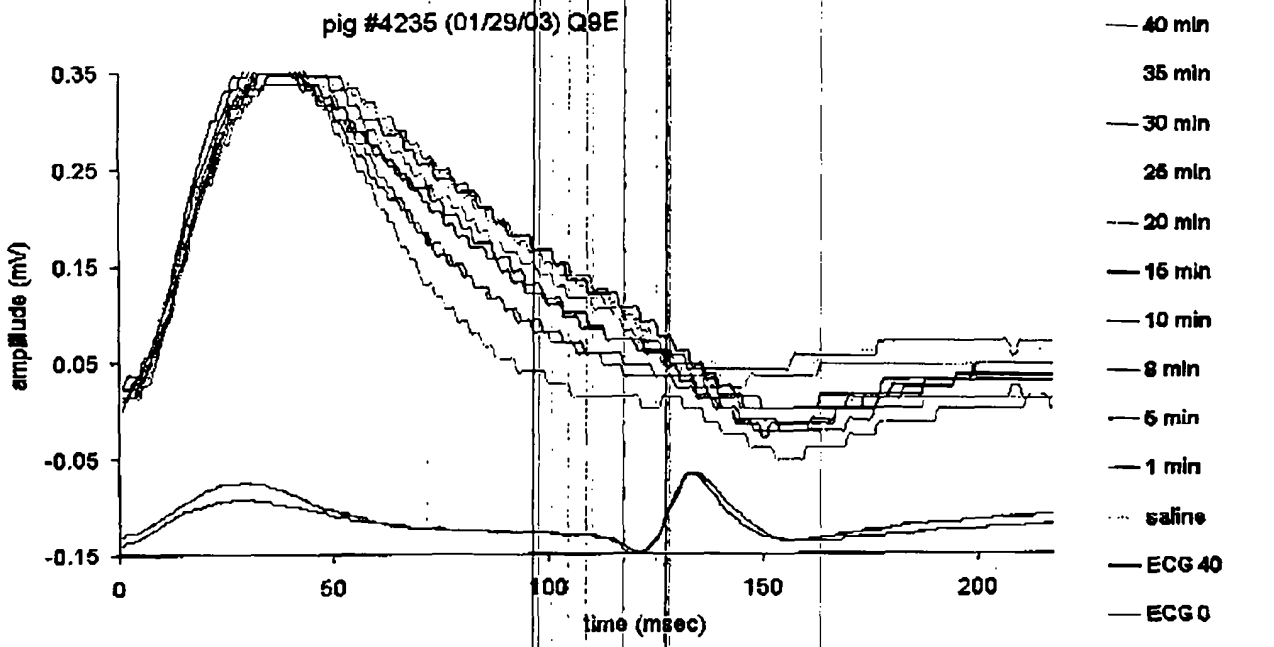
- Electrophysiologic effects comparable to class III anti-arrhythmic agents, that are commonly used to treat atrial and ventricular arrhythmias
- EP effects modulated by clarithromycin administration
- Atrial expression would be localized, and would not be expected to be associated with ventricular pro-arrhythmic effects


The Potassium Channel.
4 pore-forming proteins
(α subunits)
"accessory"
β subunit
exterior
interior
P
Wild-type
Channel,&
its mutant,
Q9E,used in
this study
α- subunit
MiRP
Examples:
KCNQ1
MinK
HERG
MiRP1
Kv3.4
MiRP2
Abbott & Goldstein, *Mol. Intervent.* (June 2001)

Methods:

1. Creation and characterization of bi-cistronic plasmid DNA vectors for MiRP-1 & Q9E
2. Establish stable cell lines (HEK293) overexpressing the vectors (using antibiotic selection w gentamycin)
3. Single cell electrophysiology (patch clamp) studies
4. Large animal (pig) studies: plasmid DNA injection to the right atrium with endpoints of expression and electrophysiologic changes (monophasic action potential, MAP).

#4235 Q9E



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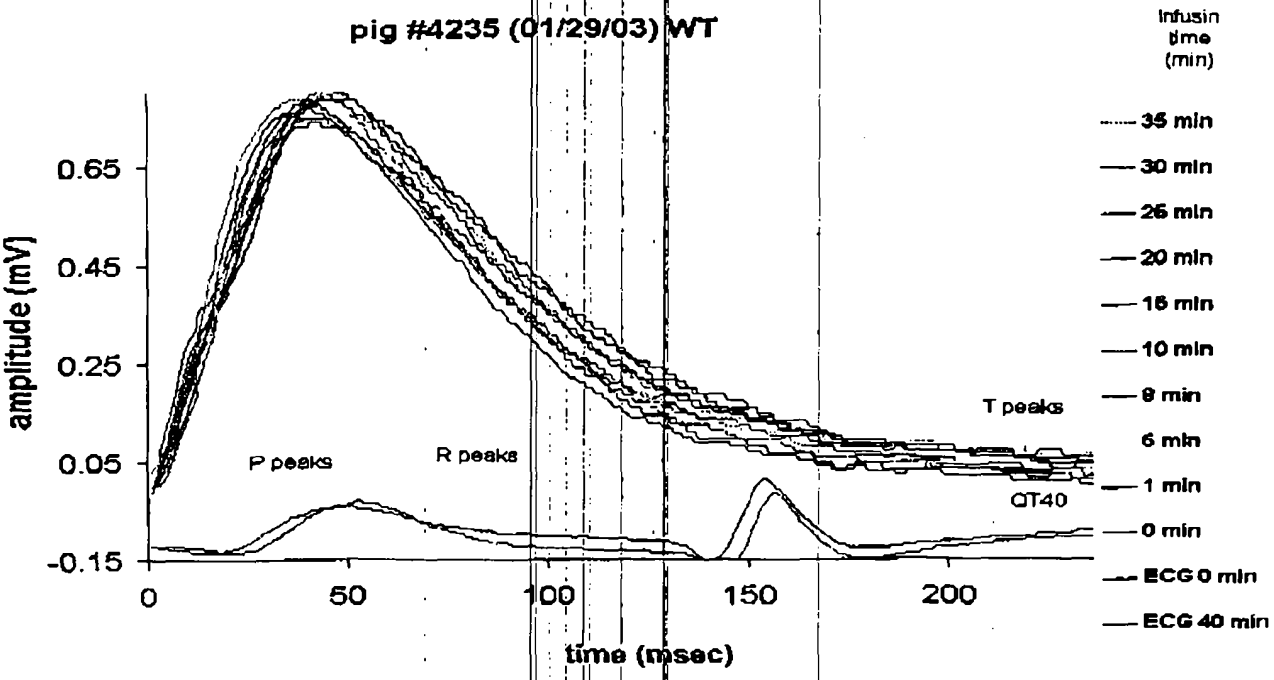
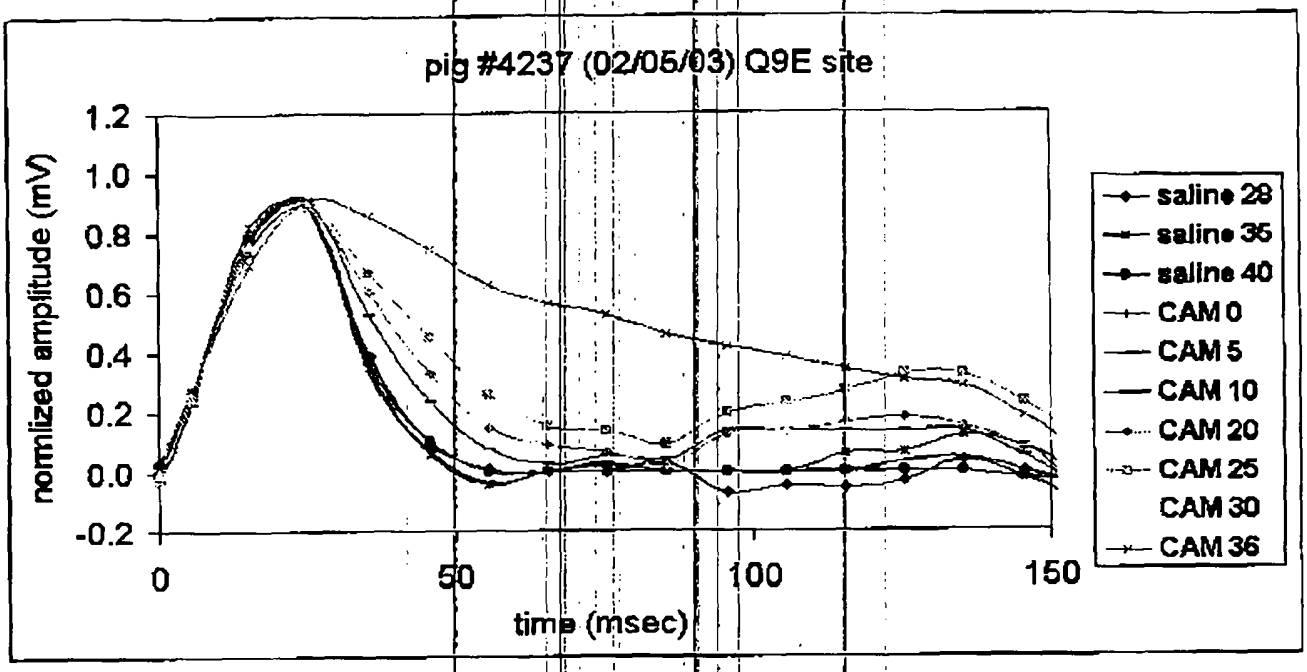


#4237 Q9E



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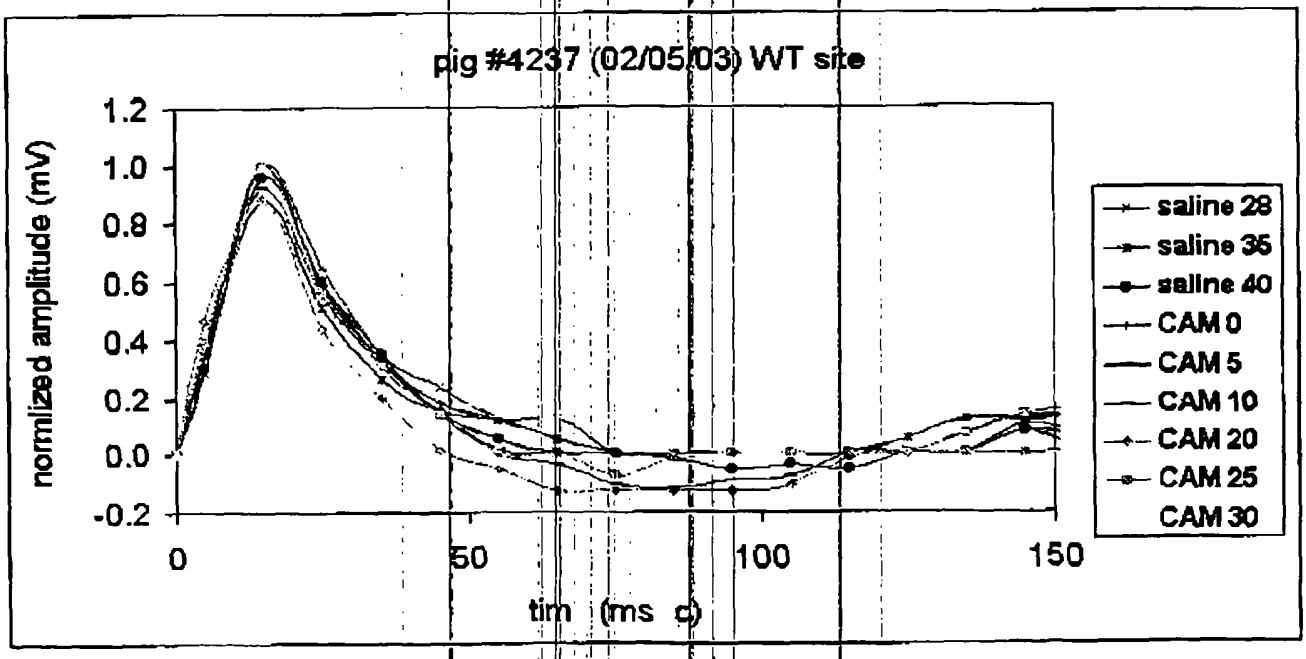
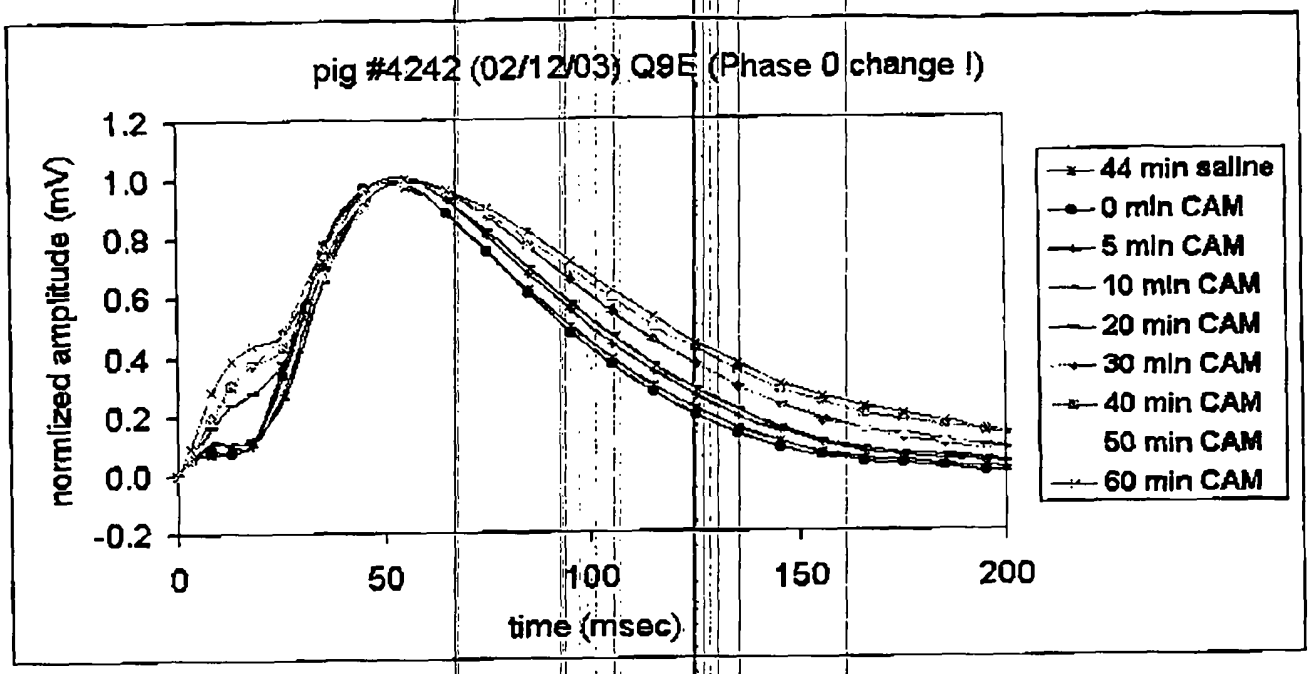


#4242 Q9E



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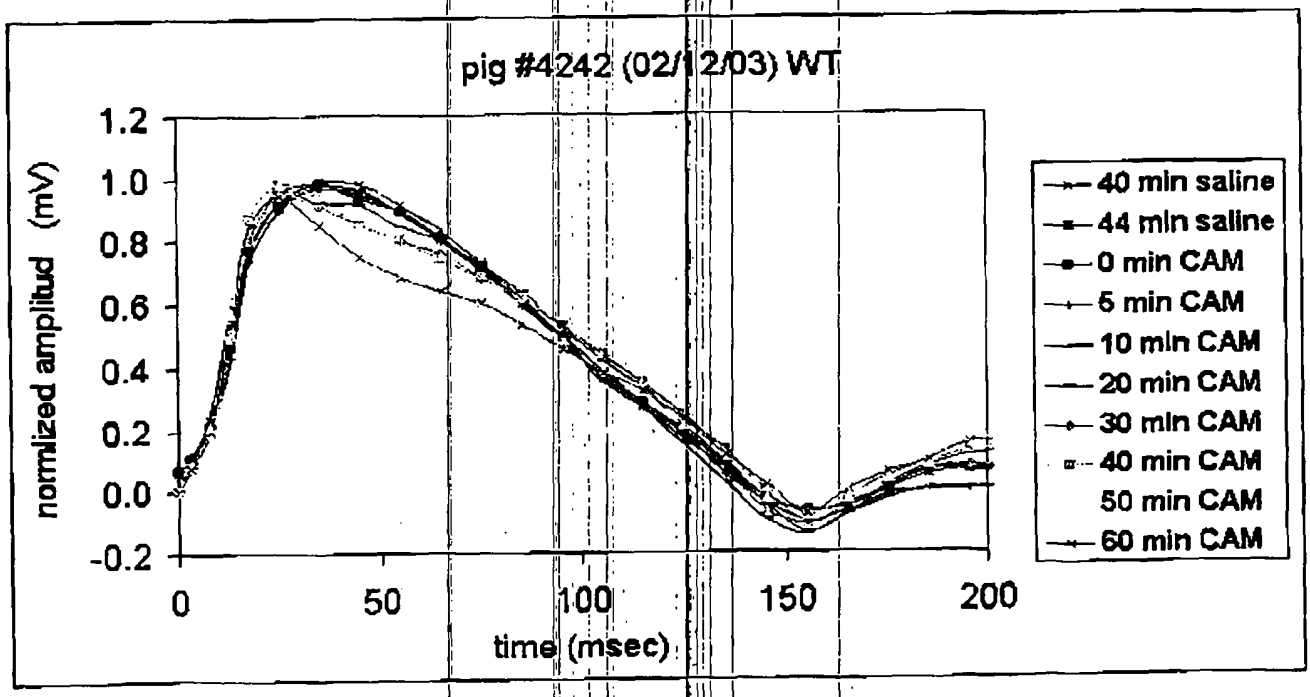
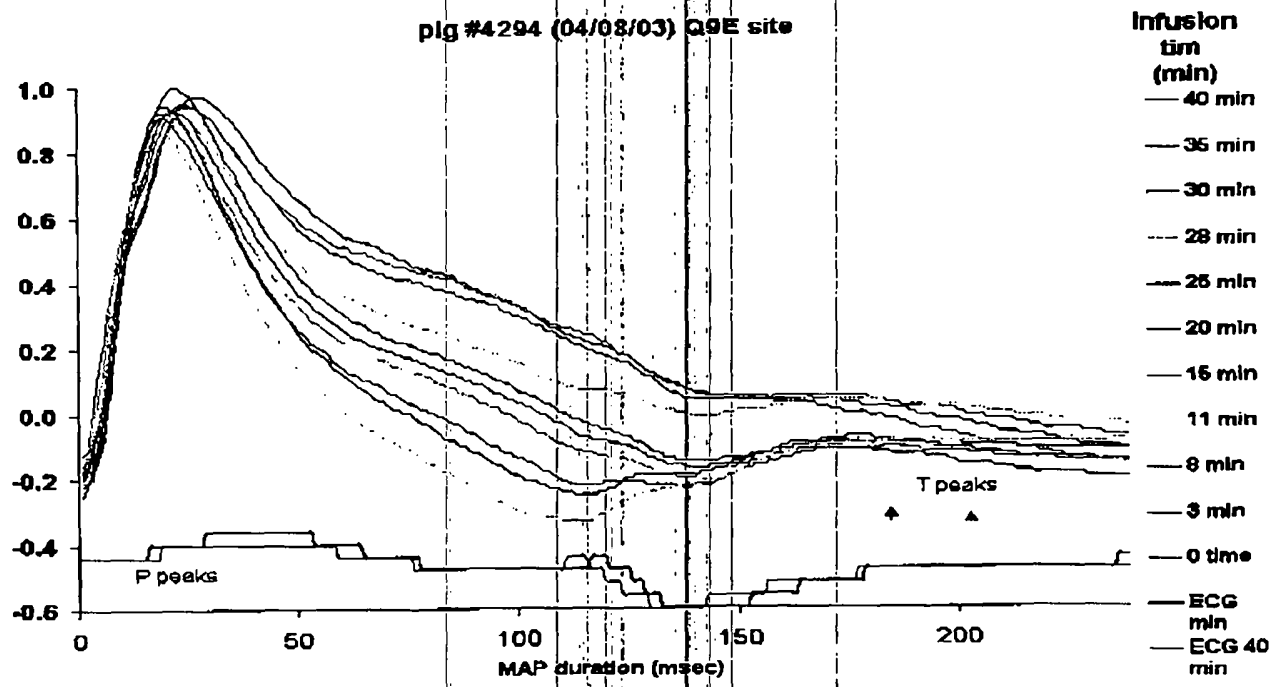


#4294 Q9E



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